PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G3126PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/005936	International filing date *(day/month/year)* 02.06.2004	Priority date *(day/month/year)* 02.06.2003
International Patent Classification (IPC) or national classification and IPC C12N15/74		
Applicant B.R.A.I.N. BIOTECHNOLOGY RESEARCH AND ..., et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.12.2004	16.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Seranski, P Telephone No. +49 89 2399-7846

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/005936

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-23	as originally filed
Claims, Numbers	
1-20	received on 02.11.2004 with letter of 02.11.2004
Drawings, Sheets	
1/16-16/16	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D2: STEDMAN KENNETH M ET AL: "Genetic requirements for the function of the archaeal virus SSV1 in Sulfolobus solfataricus: Construction and testing of viral shuttle vectors" GENETICS, vol. 152, no. 4, August 1999 (1999-08), pages 1397-1405, XP002292796 ISSN: 0016-6731

D2 represent the closest prior art. The document describes a sulfolobus expression vector which is designated as pPKMSW72. The vector is derived from the vector pKMSD, based on the SVV1 virus and contains the sulfolobus beta-galactosidase gene lacS reporter gene. The document discusses the importance of the viral integrase gene and the use of the Tind promotor for regulated expression or over expression of genes in S.solfactoricus.

The subject matter of independent claim 1 differs in that the vector of the present application should contain one or more selectable markers gene(s) encoding an essential protein of sulfolobus, operatively linked to sulfolobus expression control sequences.

The use of the selectable markers genes(s)encoding an essential protein of sulfolobus results in a stable transformation of Sulfolobus.

The objective technical problem solved by the present application can thus be formulated as the provision of a novel sulfolobus expression vector system which provides for means and methods for a stable transformation of Sulfolobus.

Said technical problem has been solved by the provision of a Sulfolobus expression vector according to claim 1. The essential technical feature by which the Sulfolobus expression vector of the present application can be distinguished from sulfolobus vectors known in the art is that the present vector comprises one or more selectable markers genes which encode an essential protein of sulfolobus. In contrast, transformation of Sulfolobus with the vector pKMSW72 known from D2 does not result in a stable transformation of the bacteria.

Consequently, present independent claim 1 and all claims 2-20 that are all related to the sulfolobus expression vector as claimed in claim 1 fulfil the requirement of inventive step (Art.33(3) PCT).

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CLAIMS

1. A sulfolobus expression vector comprising:
   (a) a sulfolobus origin of replication;
   (b) the genes encoding the structural proteins and the site-specific integrase of SSV1, SSV2 or pSSVx, operatively linked to expression control sequences and a packaging signal;
   (c) one or more selectable marker gene(s) encoding an essential protein of sulfolobus, operatively linked to sulfolobus expression control sequences; and
   (d) a sulfolobus promoter followed 3' by a restriction enzyme recognition site or a multiple cloning site for insertion of a gene of interest and optionally a 3' regulatory element.

2. The expression vector of claim 1, wherein the origin of replication of (a) is selected from the group consisting of SSV1, SSV2, pSSVx and pRN plasmids.

3. The expression vector of claim 1 or 2, wherein the vector contains the complete genome of SSV1, thereby providing said origin of replication, said packaging signal and said genes encoding the structural proteins and the integrase of SSV1.

4. The expression vector of claim 3, wherein the essential gene is a gene of the de novo nucleotide anabolism, a gene of the aminoacid biosynthesis or a gene conferring antibiotic resistance

5. The expression vector of anyone of claims 1 to 4, wherein the vector contains orotidine-5'-monophosphatase pyrophosphorlyase and orotidine-5'-monophosphatase decarboxylase as selectable marker genes.

6. The expression vector of any one of claims 1 to 5, wherein the vector contains 3' to the translation initiation site of the promoter for the expression of the gene of interest additional nucleic acid sequences so that the expressed protein has an N-terminal extension.

7. The expression vector of claim 6, wherein the N-terminal extension is
(a) a signal sequence directing the secretion of the expressed protein;
(b) a tag for purification; or
(c) a tag for specific detection.

8. The expression vector of any one of claims 1 to 7, wherein the promoter for the expression of the gene of interest is a constitutive promoter selected from the group consisting of genes involved in central metabolisms and information processing including the promoters of the ribosomal subunits 16S, 23S rRNA or the promoters of polymerases, transcription, replication or translation factors.

9. The expression vector of any one of claims 1 to 8, wherein the promoter for the expression of the gene of interest is an inducible promoter.

10. The expression vector of claim 9, wherein the inducible promoter is selected from the group consisting of (a) heat inducible promoters Tf55alpha, TF55beta, TF55gamma, hsp20, htrA, (b) cold inducible promoters TF55gamma and (c) promoters inducible by a carbon source.

11. The expression vector of any one of claims 1 to 10, wherein the vector contains an additional expression cassette for a reporter protein, selected from the group consisting of ß-galactosidase, luciferase, green fluorescent protein and variants thereof.

12. A shuttle vector comprising the sequences of the expression vector of any one of claims 1 to 11 and additional sequences for propagation and selection in E. coli, wherein the additional sequences comprise
    (a) an E.coli ori of replication; and
    (b) a marker for selection in E.coli.

13. The shuttle vector of claim 12, wherein the marker of selection is selected from the group consisting of ampicillin, kanamycin, chloramphenicol, tetracyclin, hygromycin, neomycin or methotrexate.

14. A host cell transformed with the expression vector of any one of claims 1 to 13, wherein the host cell is E. coli or sulfolobus.

15. The host cell of claim 14, wherein the transformed expression vector provides a gene encoding an essential protein.

16. The host cell of claim 14, wherein the host is deficient in expressing a fully functional version of said essential gene provided by the expression vector.

17. A method of producing a polypeptide comprising culturing the host cell of any one of claims 14 to 17 under suitable conditions and isolating said (poly)peptide from the cells or the cell culture supernatant.

18. A method of generating infectious recombinant subviral particles composed of the structural proteins of SSV1 and/or SSV2, having packaged the DNA of the expression vector of any one of claims 1 to 13, wherein the method has the steps of
    (a) introducing the DNA of the expression vector and the DNA of SSV1 or SSV2 into a host cells;
    (b) incubating the cells for time and under conditions sufficient to allow replication of SSV1 or SSV2 and spreading in the cell culture;
    (c) harvesting the cell culture supernatant or the host cells.

19. Use of the vector of any one of claims 1 to 13 for gene silencing by expression of RNAi or antisense RNA, wherein the vector contains a Sulfolobus promoter for transcription of a gene or parts of a gene either in antisense or sense orientation or in both orientations.

20. A kit comprising

(a) the vector of any one of claims 1 to 13,

(b) the host cell of any one of claim 14 to 16, and/or

(c) a host cell deficient in the expression of the essential protein of the vector of (a).

in one or more containers.